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(54) Title: COMPOSITIONS AND METHODS FOR THE CONTROL, DIFFERENTIATION AND/OR MANIPULATION OF PLURIPOTENT CELLS THROUGH A GAMMA-SECRETASE SIGNALING PATHWAY

(57) Abstract: The current invention relates to the control and/or manipulation of the gamma-secretase signaling pathway in pluripotent cells to stabilize the cells in a pluripotent state and/or to control the differentiation of the pluripotent cells towards a differentiated state. The invention further includes feeder layers that contain or express ligands or other compounds that inhibit gamma-secretase or Notch signaling to enhance the maintenance of pluripotent cells in a pluripotent state. The invention also includes cell culture compositions that comprise pluripotent cells and inhibitors of gamma-secretase, or activators or inhibitors of Notch signaling.

WO 2004/090110 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/09817

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 15/00, 15/63
US CL : 435/325, 320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/325, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2003/0022251 A1 (FUCHS et al) 30 January 2003 (30.01.2003), par. 0058, page 5 bridging page 6.	1-6, 9-25, 34, 36, 39, 46-51, 54-60
Y	US 2002/0119565 A1 (CLARKE et al) 29 August 2002 (29.08.2002), paragraphs 0064, and 0071.	1-6, 9-25, 34, 36, 39, 46-51, 54-60
Y	US 5,114,926 A (FRINDEL et al) 19 May 1992 (19.05.1992), column 1, lines 26-30, and lines 39-57.	1-6, 9-25, 34, 36, 39, 46-51, 54-60

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No.
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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: Please See Continuation Sheet

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claims 1-6, 9-25, 34, 36, 39, 46-51, 54-60, drawn to a cell culture composition comprising pluriipotent cells and a particularly named non-transition state analogue, *e.g.*, DAPT.

Group 2, claims 1-5, 7, 9-25, 34, 36, 39, 46-50, 52, and 55-60, drawn to a cell culture composition comprising pluriipotent cells and a particularly named transition state analogue, which is III-31-C.

Group 3, claims 1-5, 7, 9-25, 34, 36, 39, 46-50, 52, and 55-60, drawn to a cell culture composition comprising pluriipotent cells and a particularly named transition state analogue, which is L-685,458.

Group 4, claims 1-5, 7, 8-25, 34, 36, 39, 46-50, 52-53, and 55-60, drawn to a cell culture composition comprising pluriipotent cells and a particularly named transition state analogue, which is a substrate-based difluroketone peptidomimetic (DFK-167).

Group 5, claims 15-25, drawn to a cell culture composition comprising pluripotent cells and a dominant negative Notch protein.

Group 6, claims 26-33, drawn to a cell culture composition comprising pluripotent cells and Jagged-1.

Group 7, claims 26-33, drawn to a cell culture composition comprising pluripotent cells and Jagged-2.

Group 8, claims 26-33, drawn to a cell culture composition comprising pluripotent cells and Jagged-3.

Group 9, claims 26-33, drawn to a cell culture composition comprising pluripotent cells and Serrate.

Group 10, claims 26-33, drawn to a cell culture composition comprising pluripotent cells and Delta named ligand.

Group 11, claims 26-33, drawn to a cell culture composition comprising pluripotent cells and Delta-like-1.

Group 12, claims 26-33, drawn to a cell culture composition comprising pluripotent cells and Delta-like 3.

Group 13, claims 26-33, drawn to a cell culture composition comprising pluripotent cells and Delta-like-4.

Group 14, claims 26-33, drawn to a cell culture composition comprising pluripotent cells and Delta-like-homolog-1 (DLK1).

Group 15, claims 34, 36-39, drawn to a method of stabilizing human pluripotent cells, comprising the step of employing an inhibitor of a Notch protein, wherein the inhibitor is a dominant negative Notch protein.

Group 16, claims 34, 40-45, drawn to a method of differentiating human pluripotent cells, comprising the step of employing an activator of a Notch protein, wherein the activator is Jagged-1.

Group 17, claims 34, 40-45, drawn to a method of differentiating human pluripotent cells, comprising the step of employing an activator of a Notch protein, wherein the activator is Jagged-2.

Group 18, claims 34, 40-45, drawn to a method of differentiating human pluripotent cells, comprising the step of employing an activator of a Notch protein, wherein the activator is Jagged-3.

Group 19, claims 34, 40-45, drawn to a method of differentiating human pluripotent cells, comprising the step of employing an activator of a Notch protein, wherein the activator is Serrate.

Group 20, claims 34, 40-45, drawn to a method of differentiating human pluripotent cells, comprising the step of employing an activator of a Notch protein, wherein the activator is Delta.

Group 21, claims 34, 40-45, drawn to a method of differentiating human pluripotent cells, comprising the step of employing an activator of a Notch protein, wherein the activator is Delta-like-1.

Group 22, claims 34, 40-45, drawn to a method of differentiating human pluripotent cells, comprising the step of employing an activator of a Notch protein, wherein the activator is Delta-like-3.

Group 23, claims 34, 40-45, drawn to a method of differentiating human pluripotent cells, comprising the step of employing an activator of a Notch protein, wherein the activator is Delta-like-4.

Group 24, claims 34, 40-45, drawn to a method of differentiating human pluripotent cells, comprising the step of employing an activator of a Notch protein, wherein the activator is Delta-like-homolog-1 (DLK1).

Group 25, claims 1-5, 9-14, 34, 36, 39, 46-50, and 55-60, drawn to a cell culture composition comprising pluripotent cells and a helical peptide containing alpha-aminoisobutyric acid.

Group 26, claims 1-5, 9-14, 34, 36, 39, 46-50, and 55-60, drawn to a cell culture composition comprising pluripotent cells and a Fenchylamine Sulfonamide compound.

Group 27, claims 1-5, 9-14, 34, 36, 39, 46-50, and 55-60, drawn to a cell culture composition comprising pluripotent cells and a NSAID.

Group 28, claims 1-5, 9-14, 34, 36, 39, 46-50, and 55-60, drawn to a cell culture composition comprising pluripotent cells and a benzodiazepine.

The inventions listed as Group 1-28 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Each group of the listed groups is either directed to a distinct goal or a materially distinct material/step wherein a distinct composition and/or technical feature is employed for achieving an intended goal. For example, the special technical feature of Group I is an inhibitor DAPT for use in stabilizing a pluripotent cell. The special technical feature of Group 2 is the use of III-31-C. The special technical feature of Group 3 is L-685,458. The special technical feature of Group 4 is DFK-167. The special technical feature of Group 5 is a dominant negative Notch protein. The special technical feature of Group 6 is Jagged-1. The special technical feature of Group 7 is Jagged-2. The special technical feature of Group 8 is Jagged-3. The special technical feature of Group 9 is Serrate. The special technical feature of Group 10 is a Delta named ligand. The special technical feature of Group 11 is a Delta-like-1. The special technical feature of Group 12 is a Delta-like 3. The special technical feature of Group 13 is Delta-like-4. The special technical feature of Group is a Delta-like-homolog-1 (DLK1). The special technical feature of Group 25 is a aminoisobutyric acid. The special technical feature of Group 26 is a Fenchylamine Sulfonamide compound. The special technical feature of Group 27 is NSAID. The special technical feature of Group 28 is a benzodiazepine. In addition, the special technical feature of each of the method group as listed above is directed to a distinct goal and/or the use of a distinct material as claimed in a corresponding product claimed group.
As the result, each method requires a specific technical feature and/or distinct goal. Further, 37 CFR 1.475 does not provide for multiple independent products, methods of manufacture and methods of use (37 CFR 1.475(d)).

Continuation of Box III Item 4:

1-6, 9-25, 34, 36, 39, 46-51, 54-60, drawn to a composition comprising DAPT and pluripotent cells